

Cb Genotyping protocol (PCR)

Oligos

CbPCR3': (common primer in Cb exon 3 outside of targeting construct)

5'-ACCTTCTGCTTGTCTAA-3'

DH1-187-1 (intron 2 between exon 2 and targeting construct right arm, detects wt allele)

5'-GTAAGGAAGCTGTATGC-3'

DH1-43-2 (3' end of PolNeo in Cbrec2 targeting construct: detects knockout allele)

5'-ACTGTGCCTTCTAGTTG-3'

We run two different reactions. One reaction for the wt (2000 bp product) and one for the knockout (2200 bp product). We run the reaction on 1% agarose gel .

The program we run on the thermocycler is

98°x1 min followed by 35 cycles of (94° x 60 sec, 54°x90 sec, 72°x180 sec)

We typically use about 150 ng of tail digested DNA suspended in low TE (1 mM Tris, 25uM EDTA) in each (50 ul) reaction as follows:

H2O	31 ul
Buffer 9 (10X)	5 ul
BSA (1mg/ml)	5 ul
dNTP's(2 mM each)	5 ul
DTT(230mM)	1 ul
Amp Taq (5u/ul)	0.5 ul

Plus approximately 350 ng of each oligo for each reaction.

Use DH1-43-2 and CbPCR3' for the knockout reaction

Use DH1-187-1 and CbPCR3' for the wild type reaction.